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GENETICAL STUDIES ON *ENOTHERA*. II

SOME HYBRIDS OF *Enothera biennis* AND *O. grandiflora*
THAT RESEMBLE *O. Lamarckiana*¹

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THE status of *Enothera Lamarckiana* is a matter which must be given serious consideration in any attempt to judge the value of De Vries's mutation theory, for the reason that the behavior of this form in throwing off marked variants (mutants) from the type has been regarded by De Vries as direct experimental proof of mutation. Indeed, the theory of De Vries may fairly be said to rest chiefly upon the behavior of this interesting plant, the account of which forms so large a part of his work, "Die Mutationstheorie," 1901-1903.

Aside from his claim of direct proof of the origin of mutations from *Enothera Lamarckiana*, De Vries offers a considerable body of indirect evidence of the sort presented in Darwin's "Variation of Animals and Plants under Domestication," and in that extensive and very carefully sifted account of Bateson, "Materials for the Study of Variations," 1894. However, much of this indirect evidence of De Vries deals with the origin of "sports" from domesticated forms or with the origin

¹ Contribution from the Laboratory of Genetics, Bussey Institution of Harvard University No. 7. An investigation conducted with aid from the Elizabeth Thompson Science Fund for which the author desires to express his indebtedness.

of new forms under conditions that are not typical of those of nature in the wild. For these reasons such evidence could never appeal with so much force as would direct experimental proof that a wild species is in the habit of producing suddenly new types sufficiently distinct from the parent form to rank as new species or even as strongly marked varieties.

In "Die Mutationstheorie" of De Vries the behavior of *Oenothera Lamarckiana* in giving rise to the so-called mutants is presented as evidence that new species have come into existence without intermediate steps from a form which is assumed to be typical of a species in nature. *Oenothera Lamarckiana* is made to bear the weight of an elaborate hypothesis, treating of fundamental problems, very much as the apex might be made to bear the weight of an inverted pyramid. As the equilibrium of the inverted pyramid depends upon the stability of its apex, so the value for the mutation theory of the evidence from the behavior of *Lamarckiana* must rest with the status of this plant as a form truly representative of a typical species.

De Vries from the beginning took it for granted that *Oenothera Lamarckiana* was a native American species introduced into Europe, an assumption that was perhaps not unnatural, although dangerous when the responsibility of direct proof of the origin of species by mutation was laid upon its behavior. As far as the writer is aware, *O. Lamarckiana*, as a wild American species, is unknown. No American locality can be cited where it may be found as a clear component of the native flora. There are certain records of its presence under conditions that indicate the possibility of its being sometimes a garden escape, and there is some herbarium material, referred to *Lamarckiana*, which, however, has not been tested by culture and was collected at times when the importance of the most critical judgment in identification was not appreciated. It cannot be said that American botanists are not alive to the importance of the status of

Lamarckiana, for it is well known that a certain group would follow with persistence any clue that might give evidence of its being or having been an American native species.

Critics of the evidence for De Vries's mutation theory have been aware of the point of weakness that lay in the uncertain status of *Ænothra Lamarckiana* and the suggestions of Bateson and Saunders ('02, p. 153), East ('07, p. 34), Boulenger ('07, p. 363), Leclerc du Sablon ('10, p. 266), Tower ('10, p. 322), and others have probably occurred to many, namely, that this plant is of hybrid origin and that the appearance of its "mutations" is due to the continued splitting off of variants after the manner of hybrids. This view is held by a number of American botanists with whom the writer is acquainted and represents the attitude of those who are sceptical of the importance of mutation as a factor of organic evolution in nature. If *Lamarckiana* is of hybrid origin it should be possible to obtain evidence of its probable parentage, and the present paper offers a hypothesis with a considerable body of evidence in its favor. After the evidence has been presented the hypothesis will be discussed in the concluding section entitled "The Possible Origin of *Ænothra Lamarckiana* as a Hybrid of *O. biennis* and *O. grandiflora*."

None of the hybrids of *biennis* and *grandiflora* described in the following pages are identical with *Lamarckiana*. There are important differences, chiefly of foliage and stem markings, which distinguish the hybrids at a glance, but on the other hand these characters in taxonomy would be considered of minor importance and the hybrids, if their origin were unknown, could not be placed elsewhere than next to *Lamarckiana*. Furthermore, these differences are of a sort that are likely to be much less apparent when the results of crosses made this summer (1910) between certain types recently differentiated become known in succeeding cultures. In an investigation of this character the results, as every

student of genetics knows, come slowly, and the writer feels no necessity of offering an apology in publishing preliminary data, since they are based on three seasons' study, even though he hopes to present more conclusive evidence in the future.

The cultures of the past season (1910) were grown partly at the Bussey Institution and partly in the Botanic Garden of Harvard University, where facilities were offered for which the writer is deeply indebted.

METHODS

To break the biennial habit and obtain flowering plants in one season it is only necessary to sow seeds in the hot house during the winter, where rosettes will develop, which may be set out in the open in the early spring. The cultures of 1910 were sown early in January and had developed large rosettes by May 5, when the plants were transferred to the gardens. It is best to sow the seeds thinly in large seed pans (with, of course, sterilized soil) from which each individual seedling may be potted.

Cross pollination was always performed on flowers from which the unopened anthers had been removed. The best results are generally obtained when a castrated flower is left bagged for twelve to twenty-four hours before the pollen is applied, in order that the stigma may have time to mature fully which will be evident from the moist exudation on its surface. Strong manila bags tied firmly over the pollinated flowers have proved more satisfactory than special bags of paraffin paper, which appear to hold the flowers in an atmosphere too moist for the best results. It is my practise to dip the forceps in a bottle of alcohol before each transfer of pollen and also to rinse the hands in alcohol. The pollen of *Enothera* is so sticky that under ordinary conditions there is no danger from wind blown pollen, and by following the practise outlined above there is little or no probability of impure pollination.

In the future my plan will be to sow the seed capsule

by capsule, which is the safest method to obtain quantitative results. In the past I have sown from bulk collections of seed and my studies so far must be regarded as primarily qualitative in character. This practise was followed under the impression that probably only a small proportion of the seed from a cross, apparently as extreme as that between *biennis* and *grandiflora*, would germinate. The results, however, have shown that the seed of this cross is fertile to a very high degree. In consequence my cultures of this season gave three or four times more seedlings than it was practicable to bring to maturity. A process of selection became at once necessary, which was followed with the end in view of obtaining a variety of types, but it soon became evident, with the later development of the cultures, that it is impossible to select with accuracy among the young seedlings. Thus plants which showed certain tendencies as young rosettes or seedlings of five to nine leaves often changed very materially in later growth. For these reasons my cultures of this year even as qualitative studies are undoubtedly not fully representative.

Ænothera biennis L.

My first crosses between *biennis* and *grandiflora* were made at Woods Hole, Mass., in 1908. This locality was carefully searched for rosettes of *biennis* with broad leaves and two plants of this character were transplanted from waste ground to the garden. The rosettes were similar and at maturity the plants proved to be the same form of *biennis* and were practically indistinguishable. These two plants were the starting points of two strains of *biennis*, designated A and B, which have been cultivated through two and three generations, respectively, and have proved constant.

It seems quite certain that under the name *Ænothera biennis* is included a number of races with well-marked peculiarities. These races are probably very pure, for the reason that close pollination is certainly usual, if

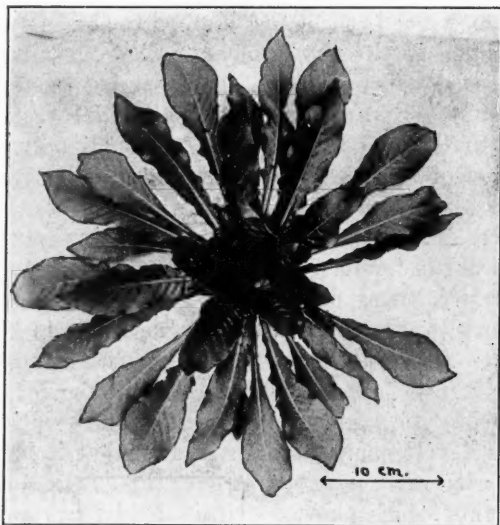


FIG. 1. Mature rosette of *Enothera biennis*, B (10.21a).

not universal, among the forms of the species. The lower portions of the stigma lobes in the bud lie below the tips of the anthers which discharge their pollen before the bud opens. As a result the stigma is not only well pollinated in the bud, but cytological studies on my strains A and B have shown that the pollen tubes reach the embryo sacs before there is any opportunity for cross pollination. Hybridization in nature could hardly occur in these forms unless their own pollen should be insufficient for the number of ovules in the ovary or was much slower than foreign pollen in effecting fertilization; alternatives that are very unlikely. These conditions, similar physiologically to those of cleistogamous flowers, are present in all of the forms of *biennis* known to the writer, and have been noted by De Vries for the European types. As a result a strain once established is certain to remain pure throughout at least the great mass of its seeds.

The chief characteristics of the *biennis* strains A and



FIG. 2. Mature plant of *Enothera blennis*, B (10.21a).

B, employed in the hybrid studies of the present paper, when under good cultivation are as follows:

1. *Rosettes*.—The mature rosettes (Fig. 1), from 3 to 4 dm. broad, have about 40 closely clustered leaves, spatulate, irregularly toothed at their base, and green except for occasional reddish spots. The rosettes are persistent and conspicuous during the early development of the main stem.

2. *Mature Plants*.—The mature plants, 1–1.5 m. high, have a spreading habit (Fig. 2) with long side branches. Stems chiefly green (brownish below), the papillate glands at the base of long hairs also green. Basal leaves on the main stem narrowly elliptical, about 16 cm. long (Fig. 3), leaves above lanceolate.

3. *Inflorescence*.—Bracts lanceolate, less than half the length of the buds (Fig. 4).

4. *Buds*.—About 5.5 cm. long, the cone 4-angled (Fig. 4). Sepals clear green, their tips not markedly attenuate.

5. *Flowers*.—Small (Figs. 3 and 5). Petals about 1.3 cm. long. Lower halves of stigma lobes (when closed) below the tips of the anthers.

6. *Capsules*.—Gradually narrowing from the base, 2–2.5 cm. long.

7. *Seeds*.—Light brown. Those developed in the

ovary of *biennis* after pollination by *grandiflora* are similar to the female parent.

The most striking peculiarities of strains A and B in comparison with certain other American types of *biennis* are:—the small flowers, green stems, and the absence of that red coloration in the papillate glands which is seen in some forms of the species and is conspicuous in *Lamarckiana*.

Since differentiating the strains A and B in 1908, I have had an opportunity of observing somewhat extensively various forms of *biennis* in the vicinity of Boston, where the prevailing type has larger flowers, frequently a broader leaf, and usually

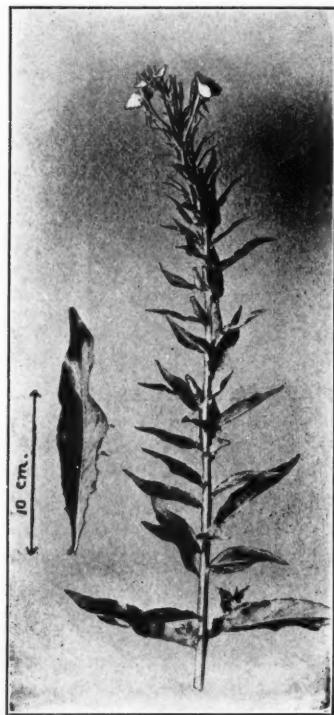
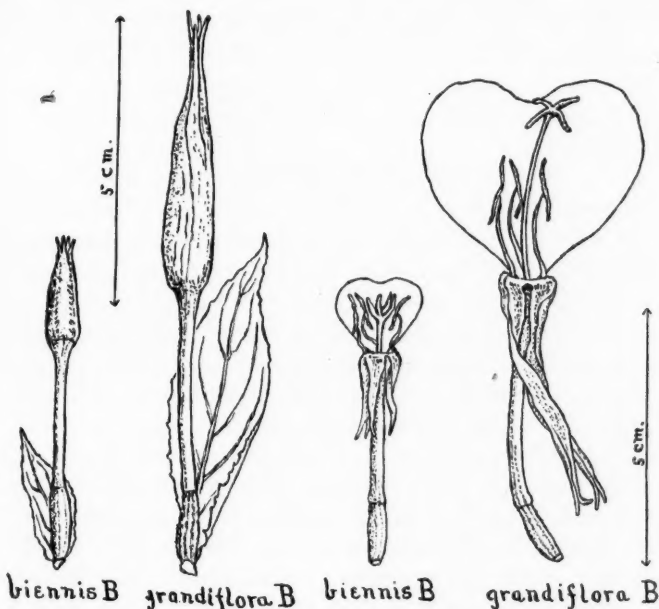


FIG. 3. Flowering side branch of *Enothera biennis*, B (10.21a), with a leaf from lower portion of main stem.

stems punctate with red tinted glands. There is considerable variation in the characters above mentioned and I have this year selected certain plants growing wild on the grounds of the Bussey Institution that in some respects are likely to prove much more satisfactory for my purposes than the strains A and B. These

FIG. 4. Buds of *biennis* B and *grandiflora* B.FIG. 5. Flowers of *biennis* B and *grandiflora* B.

plants (strains C and D) have been crossed this year with the best strains of *grandiflora* (B and D) and I expect to grow the hybrids in another season, when the strains will also be tested for their stability. The testing for purity is of course a necessary precaution, although, as explained above, the habit of self-pollination in *biennis* makes it very unlikely that any of these plants are tainted with foreign blood. These strains will not be further described until the prospective cultures have been grown.

Sowings from the wild seed collected as *Ænothera grandiflora*, described below, have given several plants of a southern form of *biennis* (strain S) which may prove of considerable interest since some of its characters (stem coloration, form of buds, size of flowers, etc.) are

very favorable for combination with *grandiflora* with certain ends in view. This southern strain was crossed this season with *grandiflora*, and the hybrids and the parent *biennis* will be studied through further cultures.

Oenothera grandiflora Ait.

We are indebted to Dr. MacDougal ('05, p. 7) and to Miss Vail ('05, p. 9) for accounts of the rediscovery of this remarkable species of the southern United States and for a clear analysis of its probable history. Aiton's original description (1789), from material grown at Kew, states that *Oenothera grandiflora* was introduced by John Fothergill, M.D., in 1778. An expedition of William Bartram in 1776, undertaken at the request of Dr. Fothergill for the purposes of botanical discovery, records the finding of a large-flowered *Oenothera* near Tensaw (Taensa), Ala. Bartram's picturesque and excellent description of this new plant (see MacDougal, '05, p. 7), together with a herbarium specimen in the British Museum from "Hort. Fothergill, 1778," makes it evident that Bartram must have sent seed to Fothergill, through whom the plant was introduced into England in 1778.

Professor S. M. Tracy in 1904 visited the original locality and found the species growing in considerable quantity along the east bank of the Alabama river in the vicinity of Dixie Landing, which is only a few miles from Tensaw. His material agrees with the descriptions of Bartram and Aiton and, according to Miss Vail, with the herbarium specimen of Fothergill, and there seems to be no doubt that *Oenothera grandiflora*, so widely cultivated, has been rediscovered growing wild in its original locality.

My seed of *Oenothera grandiflora* was collected by Professor Tracy at Dixie Landing in September, 1907. During the past three seasons I have sampled the collection to the extent of bringing to maturity from the wild seed thirty-four plants, and in addition some seventy rosettes and young plants have passed under my

inspection. Besides the above I have selected and cultivated from this material three strains (*grandiflora* A, B, and D), which have been carried through a second generation represented by twenty-four mature plants.

It became apparent, as my cultures progressed, that the *Enothera grandiflora* growing near Tensaw is far from uniform in character. I have so far selected four distinct types of plants, only one of which, however, has been cultivated in later generations. The fact that the stigma of *grandiflora* is generally well above the tips of the anthers prevents pollination in the bud and in the opening flower, and offers a very much greater opportunity for cross pollination than is possible in *biennis*. This condition is probably responsible for the heterogeneity of the species.

The type which I have under cultivation and which has proved stable is characterized by a somewhat broader leaf than is common to the species. From it have been differentiated the following three strains (A, B, and D), which have been used in the crosses with *Enothera biennis*.

1. Strain D came directly from a wild plant.
2. Strains A and B are the result of a cross between D and a similar plant, F, in the garden at Woods Hole in 1908. The cross was made to fix thoroughly the characters of a broad leaf and red coloration of sepals present in both parents. The two strains (A and B) are essentially similar, differing chiefly in the degree of red coloration present in the sepals, a character that is variable and probably cannot be depended upon as a fixed factor. As a matter of fact, strain D in a second generation has proved very uniform and it is not likely that I shall make further use of strains A and B. All three strains are representative of the larger proportion of the plants of *grandiflora* that have passed under my observation, being, however, what a gardener would select as luxuriant forms with broader and larger leaves than the average.

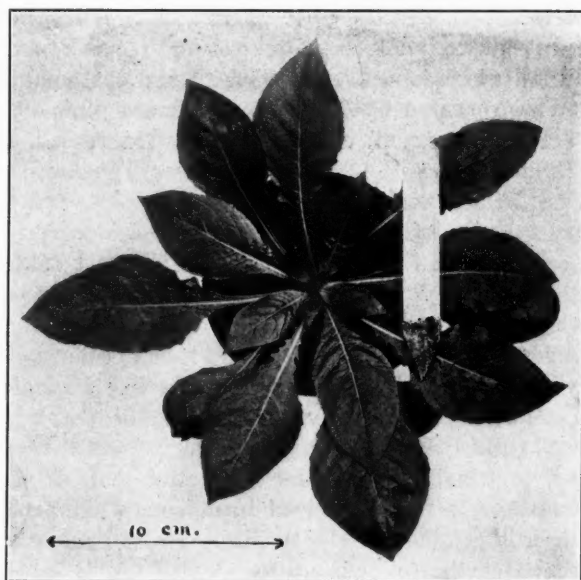


FIG. 6. Mature rosette of *Enothera grandiflora*, B (10.4a).

In addition to the type represented by the strains A, B, and D (which is described in the paragraph below) there have appeared in the cultures from the wild seed the following three types markedly different from one another and from the general run of wild *grandifloras*.

1. A type represented by a large plant (2 m. high) peculiar for its light green broadly elliptical leaves, generally green stem, green sepals, and a close rosette of crinkled leaves strongly resembling a half-grown rosette of *Lamarckiana*. This type, represented by a single plant (*grandiflora* I) appeared this year, 1910, and is likely to prove of great interest. It will not, however, be described until its behavior in later generations has been noted and its crosses with *biennis* have been grown.

2. A type represented by a relatively small plant (1.2 m. high) with stiff, broadly lanceolate, revolute leaves, and sepals deeply blotched with red. This peculiar form



FIG. 7. Mature plant of *Ænothera grandiflora*, B (10.4c).

(*grandiflora* R) is too far from the general type of *grandiflora* to be of value in the present study.

3. A type represented by a plant (*grandiflora* Z) with narrow lanceolate leaves, much too narrow to give results, if crossed with *biennis*, that would approach *Lamarckiana*.

The chief characteristics of the *grandiflora* strains A, B, and D, employed in the hybrid studies of the present paper, when under good cultivation are as follows:

1. *Rosettes*.—The mature rosettes (Fig. 6), 2–2.5 dm. broad, have about 20 loosely clustered leaves, broadly elliptical, irregularly and sometimes deeply cut at the base, slightly crinkled, and mottled with reddish brown blotches. The rosettes are transitory, the leaves withering during the development of the main stem.

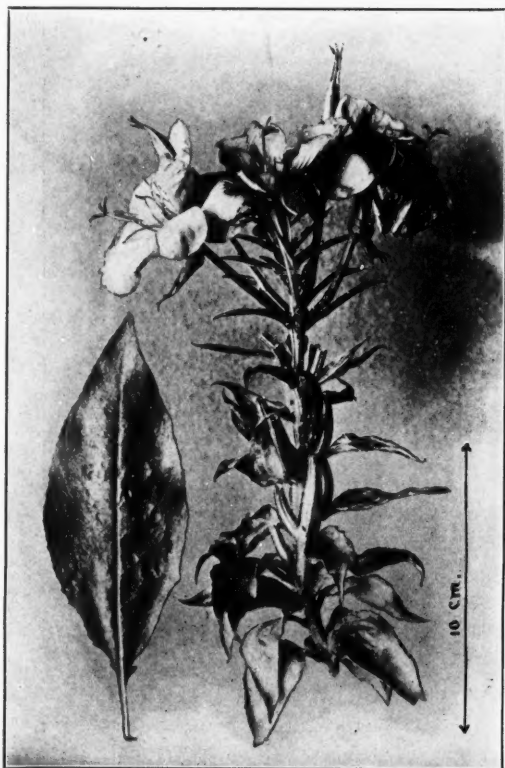


FIG. 8. Flowering side branch of *Oenothera grandiflora*, B (10.4a), with a leaf from the lower portion of the main stem.

2. *Mature Plants*.—The mature plants (Fig. 7), 1.5–2 m. high, have a more upright habit than *biennis*. Frequently the branching is profuse, the main stem and long side branches being covered with short shoots. Stems reddish, green only towards their tips, the papillate glands following the color of the stem. Basal leaves on the main stem ovate or broadly elliptical, about 15 cm. long (Fig. 8); leaves above broadly lanceolate.

3. *Inflorescence*.—Bracts early in the season leaf-like and more than half the length of the buds (Fig. 4); later becoming very much smaller.

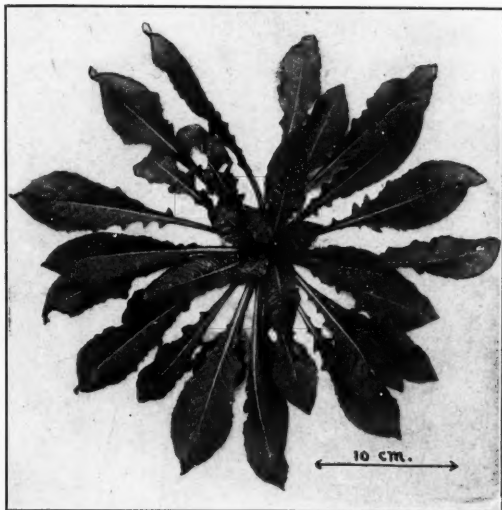


FIG. 9. Rosette of a hybrid (10.30 La), *grandiflora* B \times *biennis* A,
F₁ generation.

4. *Buds*.—From 9–10 cm. long, the cone scarcely angled (Fig. 4). Sepals marked with reddish brown blotches, sometimes dull and faint; their tips attenuate.

5. *Flowers*.—Large (Figs. 5 and 8). Petals about 3.3 cm. long. Stigma lobes generally 2–5 mm. above the tips of the anthers.

6. *Capsules*.—Tapering rather sharply from the large base, 2.5–3 cm. long.

7. *Seeds*.—Dark brown. Those developed in the ovary of *grandiflora*, after pollination by *biennis*, have the same color as the female parent.

The above description is so worded as to emphasize the characters of the *grandiflora* strains A, B, and D in contrast to the *biennis* strains A and B. It should be compared with the description of the latter forms to understand clearly the conditions that appear in the hybrids, which will now be described.



FIG. 10. Mature plant of a hybrid (10.30 La), *grandiflora* B \times *biennis* A, F_1 generation.

HYBRIDS IN THE F_1 GENERATION

The cultures of 1910 in the F_1 generation were hybrids of three different combinations of parents, as follows:

1. *grandiflora* B \times *biennis* A (10.30), grown at the Bussey Institution.

2. *grandiflora* B \times *biennis* B (10.18) and the reciprocal cross (10.19), grown at the Bussey Institution.

3. *grandiflora* A \times *biennis* B (10.17) and its reciprocal cross (10.20), grown at the Botanic Garden of Harvard University.

Bearing in mind that the *biennis* strains A and B are practically indistinguishable and that the *grandiflora* strains A and B are essentially similar, the cultures as a whole would not be expected to differ markedly, which was the case.

The figures of hybrids published with this paper are from two plants in the first culture (*grandiflora* B \times



FIG. 11. Flowering side branch of a hybrid (10.30 La), *grandiflora* B \times *biennis* A, F_1 generation. At the left is a leaf from the lower portion of the main stem.

biennis A). They were selected as likely to prove the most interesting for further cultures in the F_2 and later generations. These two plants 10.30 La and 10.30 Lb will be described in some detail, together with the general features of the cultures.

1. *grandiflora* B \times *biennis* A (10.30). This culture gave about 400 seedlings from which 57 were selected for the breadth of the cotyledons and the shortness of their petioles. These were brought to maturity. The characters of the parents were blended in the rosettes which had long, broadly elliptical leaves, toothed below, and col-



FIG. 12. Rosette of a hybrid (10.30 Lb), *grandiflora* B \times *biennis* A, F_1 generation.

ored with large reddish spots and blotches. The mature plants likewise presented the characters of both parents blended in the habit, foliage, and flowers, all the characteristics of form and measurements being clearly intermediate. It was possible to distinguish certain rosettes as being more *biennis*-like or more *grandiflora*-like than the culture in general and the mature plants from these rosettes also exhibited similar differences. The plants of the culture therefore presented a certain range, the extremes being readily distinguished as more like one parent than the other although never approaching closely to either. Between the extremes were numerous transitions.

Two rosettes of this culture were selected for their resemblance in certain particulars to *Oenothera Lamarckiana* and the mature plants from these proved to be among the most interesting in the gardens. These hybrids, 10.30 La and 10.30 Lb, were representative of a



FIG. 13. Mature plant of a hybrid (10.30 Lb), *grandiflora* B \times *biennis* A, F₁ generation.

type of hybrid of *biennis* and *grandiflora* that has proved not uncommon in my cultures. The description of these two plants will now follow, arranged to bring out the salient features in comparison with the parent species and with *Lamarckiana*.

Hybrid 10.30 La. 1. *Rosette*.—The mature rosette (Fig. 9), about 3.3 dm. broad, was persistent. Older leaves more elliptical and less spatulate than those of *biennis* (Fig. 1), deeply cut at the base as in *grandiflora* (Fig. 6), spotted with red. The younger leaves were markedly crinkled as in *Lamarckiana* (Fig. 15), but narrower and more pointed.

2. *Mature Plant*.—The mature plant (Fig. 10), 1.8 m. high, had a straggling habit with long branches arising from the base, short clustered shoots above (*grandiflora*-like), a habit very different from the symmetry of *La*-



FIG. 14. Flowering side branch of a hybrid (10.30 Lb), *grandiflora* B \times *biennis* A, F_1 generation. At the left is a leaf from the lower portion of the main stem.

marckiana (Fig. 16). Stem green above, mottled red below, occasional regions where red-tinted papillate glands lay on green portions of the stem as in *Lamarckiana*. Basal leaves on the main stem (Fig. 11) elliptical, about 22 cm. long, only slightly crinkled and not so long as the much-crinkled basal leaves of *Lamarckiana* (Fig. 17). Leaves on the upper portion of the plant broadly elliptical, slightly larger than those of *Lamarckiana*.

3. *Inflorescence*.—The inflorescence (Fig. 11) was strikingly similar to that of *Lamarckiana* (Fig. 17), the bracts being of about the same size and shape.

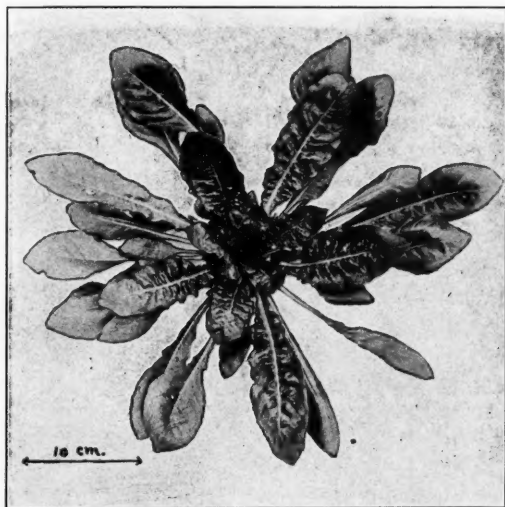


FIG. 15. Rosette of *Ænothera Lamarckiana* (10.23c).

4. *Buds*.—From 6 to 6.5 cm. long, about the same size as *Lamarckiana* and similar in form (compare Fig. 11 with Fig. 17), sepals green. Intermediate in size between those of parents.

5. *Flowers*.—Scarcely distinguishable from those of *Lamarckiana* (compare Fig. 11 with Fig. 17) and with the form and proportions of the parents blended. Petals about 2.2 cm. long. Base of stigma lobes slightly below the tips of the anthers.

6. *Capsules*.—About 2.3 cm. long, intermediate in size between those of the parents.

7. *Seeds*.—A shade of color clearly intermediate between the light and dark brown of the parents.

Hybrid 10.30 Lb. 1. *Rosette*.—The mature rosette (Fig. 12), about 3 dm. broad, was persistent. Older leaves much broader than those of *biennis* (Fig. 1), cut at the base as in *grandiflora* (Fig. 6), a lighter green than the average of the culture, and with relatively few red spots as in *Lamarckiana*. The younger leaves were



FIG. 16. Mature plant of *Enothera Lamarckiana* (10.23c).

strongly crinkled, almost as broad as those of *Lamarckiana* (Fig. 15), but more pointed.

2. *Mature Plant*.—The mature plant (Fig. 13), 1.7 m. high, with long branches from the base, had a habit more spreading than that of *Lamarckiana* (Fig. 16), but was otherwise very similar. Stems green above, mottled red and brown below, the papillate glands of the same color as the portions of the stem upon which they lay. Basal leaves on the main stem (Fig. 14) broadly elliptical, about 20 cm. long, without crinkles and not so long as the

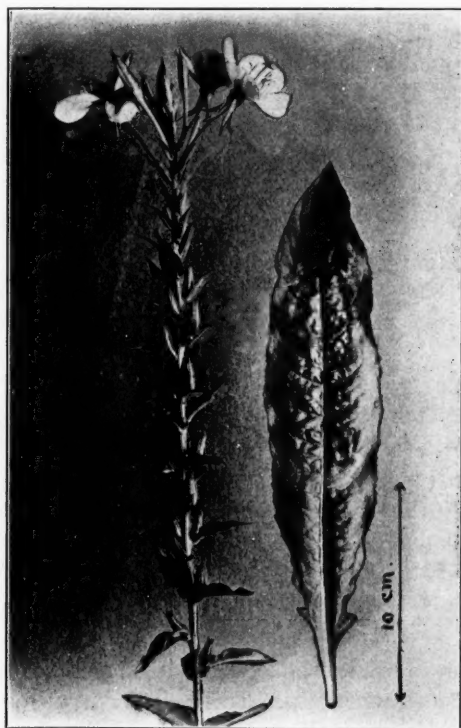


FIG. 17. Flowering side branch of *Enothera Lamarckiana* (10.23zb), with a leaf from the lower portion of the main stem.

much crinkled basal leaves of *Lamarckiana* (Fig. 17). Leaves on the upper portion of the plant broadly elliptical, slightly crinkled, similar to those of *Lamarckiana*.

3. *Inflorescence*.—The inflorescence (Fig. 14) had bracts longer than those of *Lamarckiana* (Fig. 17), somewhat crinkled and similar to the bracts in figures of *Enothera scintillans* (De Vries's "mutant" from *Lamarckiana*).

4. *Buds*.—From 6–6.5 cm. long, about the same size as those of *Lamarckiana*, but with a more pointed cone and attenuated sepal tips (contrasted in Fig. 18), sepals green. Intermediate in size between those of the parents.

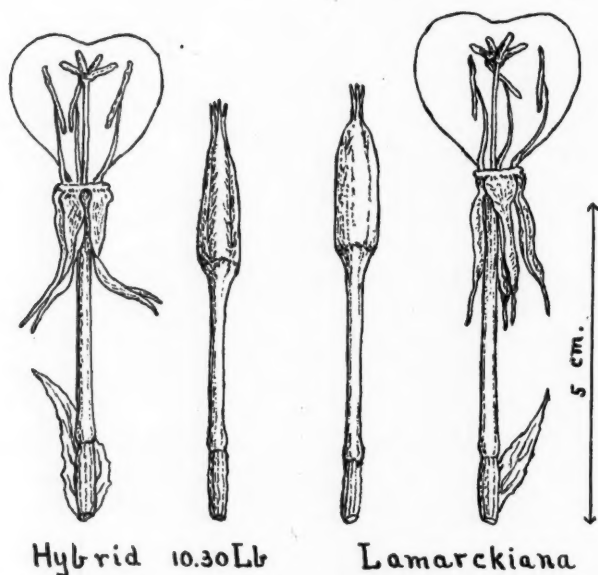


FIG. 18. Buds and flowers of a hybrid (10.30 Lb), *grandiflora* B \times *biennis* A, F_1 generation, in comparison with those of *Enothera Lamarckiana*.

5. *Flowers*.—Scarcely distinguishable from those of *Lamarckiana* (contrasted in Fig. 18), and with the form and proportions of the parents blended. Petals about 2.2 cm. long. Base of the stigma lobes slightly below the tips of the anthers.

6. *Capsules*.—About 2.5 cm. long, intermediate in size between those of the parents.

7. *Seeds*.—A shade of color clearly intermediate between the light and dark brown of the parents.

An examination of these two hybrid plants with respect to the contributions by their respective parents may be readily made by comparing the illustrations and descriptions of rosettes, mature plants, inflorescence, and flowers, bearing in mind that the parent *biennis* A is essentially indistinguishable from the strain *biennis* B which is here figured. It will be found that the hybrids present the characters of the parents in a blend. By a blend it must

not be inferred that the characters of the hybrids are a perfect mean as to the measurement and proportions of their organs. This is certainly not the case for all of the characters of the hybrids and it would be a difficult matter to determine a perfect blend for a single character because of the fluctuating variations in the parent strains. All that I desire to demonstrate in the present account is the fact of blended conditions throughout these hybrids of the first generation, and the total absence of clear dominance of one parent over the other with respect to any character.

It would be very difficult and probably impossible to support satisfactorily a claim that either of the two parent plants exhibited its influence to a measurably greater degree than the other. To illustrate this point let us examine hybrid 10.30 La. The rosette (Fig. 9) of this plant might be said to be more like that of *biennis* than of *grandiflora*, but the habit of the mature plant (Fig. 10) with respect to its short clustered shoots is more like the latter parent than the former, and thus two characteristic stages of the plant suggest opposite conclusions. This general balance of the influence of one parent over the other was manifest throughout the greater part of the culture, but, as previously noted, a small proportion of the hybrids was readily distinguishable as being more like *biennis* or *grandiflora* although never approaching closely to either parent form.

The chief points of resemblance between the two hybrid plants just described and *Oenothera Lamarckiana* may be briefly summarized as follows:

1. The inflorescence was very similar to *Lamarckiana*, especially in the case of hybrid 10.30 La from which many branches might have been picked that as herbarium specimens could not have been separated from a mixed and varied collection of *Lamarckiana* branches similarly prepared.

2. The only essential difference between the buds lay

in the slightly greater attenuation of the sepal tips especially in the case of the hybrid 10.30 Lb.

3. The flowers of the hybrids were scarcely distinguishable from those of *Lamarckiana*, the small differences in the measurement of parts being no greater than might be expected in any reasonably large and varied culture of *Lamarckiana*. The chief difference with respect to the flower lay in the clear green color of the ovaries of the hybrids, the glands of which were not tinted red, as is characteristic of all material of *Lamarckiana* that the writer has seen.

4. Although the capsules were somewhat longer and more pointed than in the forms of *Lamarckiana* grown by the writer, they were not so long as in certain "long-fruited races" described by De Vries, who states that the fruits of *Lamarckiana* are highly variable and figures capsules as pointed as those of my hybrids (De Vries '09, p. 528, et seq., Fig. 114).

The points of difference concern chiefly the vegetative portions of the plants under discussion.

1. The rosette of the hybrids consisted of mixed forms of leaves, only the younger resembling the markedly crinkled leaves of *Lamarckiana*.

2. The habit of the mature hybrid plants was more straggling, lacking the symmetry characteristic of *Lamarckiana*. The basal leaves were not so large and were but slightly crinkled; the upper leaves, especially in the case of hybrid 10.30 Lb, were similar to *Lamarckiana*.

3. The coloration of the stem was green above and mottled red and brown below, in contrast to the green stems of *Lamarckiana* punctate with red-tinted papillate glands. Similar glands were present in the hybrids, but their color (portions of 10.30 La excepted) was that of the regions of the stem upon which they lay.

The hybrids, therefore, resembled *Lamarckiana* as to the inflorescence, floral parts, and fruits; they differed chiefly in certain vegetative characters and in the coloration of the stem. It remains to be seen through further

cultures which of the two, the resemblances or the differences, are more stable in inheritance and variation.

The type of *Lamarckiana* which has been compared in this paper with the hybrids of *biennis* and *grandiflora* is one with which I have been familiar for the past five years. It has been represented in my cultures by strains from seed that has come to me through three different sources, all of the seed, however, originally being derived from the cultures of De Vries. These strains have not differed materially from one another, and as grown in my small cultures have not exhibited marked variation. The rosettes and mature plants have agreed in habit and foliage with the descriptions of *Lamarckiana* in "Die Mutationstheorie." The flowers have, however, been uniformly smaller than the measurements and figures of De Vries, the petals being about 2.5 cm. long instead of measuring 3 cm. or more. In the flower structure the position of the stigma has proved more variable than one would be led to suppose by the figures and descriptions of *Lamarckiana*, the stigma generally being but slightly above the tips of the anthers or about at their level, and in some plants distinctly below.

2. *grandiflora* B \times *biennis* B (10.18), and the reciprocal cross (10.19). From about 200 seedlings of the first culture and about 250 seedlings of the second culture, 66 and 70 plants, respectively, were brought to maturity, being selected for the breadth of the cotyledon and the shortness of its petiole. As the rosettes formed there appeared much variation in the amount of anthocyan developed in the leaves, the larger proportion being marked with dull red spots and blotches, only a small number having few spots as in *Lamarckiana*. The form of the leaves likewise varied and certain of the rosettes were readily separated as being more *biennis*-like or *grandiflora*-like than the average. The plants that developed from these extreme forms of rosettes were also somewhat more like the respective parents than the mass of the culture which presented the characters of

the parents thoroughly blended in the form and proportions of habit, foliage, and flowers. Considering the cultures as a whole, there seemed to be no marked difference between the first cross and its reciprocal.

The average types of hybrids in both crosses were essentially similar and a number of types were very close to the hybrids 10.30 La and 10.30 Lb of the previously described culture. Six plants in culture 10.19 were selected for special peculiarities, but these will not be described unless their behavior in the F_2 generation should justify a detailed account.

3. *grandiflora* A \times *biennis* B (10.17), and the reciprocal cross (10.20). From about 200 seedlings of the first culture and about 150 seedlings of the second culture, 49 and 60 plants, respectively, were brought to maturity, being selected for the breadth of the cotyledon and shortness of its petiole. These cultures were grown in a stiff clay at the Botanic Garden and presented an interesting contrast to the cultures previously described which were grown in a somewhat sandy well-fertilized soil. The plants were smaller and less vigorous vegetatively, although they flowered very freely. The rosettes and mature plants presented the characteristics of the parents well blended as in the other cultures. There were also a few extreme types that resembled one or the other of the parents more closely than the average. There appeared to be no significant differences between the first cross and its reciprocal. Two plants with marked peculiarities were selected from culture 10.17 and will be carried through an F_2 generation; they will be described if their further cultivation proves of interest.

Although the evidence, not being quantitative in character, is incomplete, nevertheless the following points may be noted, at least provisionally, from these observations on F_1 generations. (1) There was no indication from these cultures of a marked preponderance of either paternal or maternal influence upon the hybrids.

(2) No character of either parent was observed to be dominant. (3) It is doubtful whether there would be any material difference between a cross and its reciprocal if each were equally vigorous. (4) Although the extreme types in the culture, approaching somewhat the respective parents, could be readily distinguished, they were connected by transitional forms and a sharp line could not be drawn between two sets of hybrids, such as have been described by De Vries ('07, '08) as "twin hybrids" and reported for crosses between the *Onagra* group and *Lamarckiana*. From observations on small cultures during the season of 1909 (Davis '10, p. 113) the writer was led to believe that "twin hybrids" might be present in this cross, but he no longer regards this as probable.

HYBRIDS IN THE F_2 GENERATION

In a recent paper (Davis '10) I described two small cultures of hybrids between *biennis* B and *grandiflora* D that were grown at the Botanic Garden of Harvard University in the season of 1909. Four of the plants of these cultures were of special interest as presenting flowers and inflorescences very similar to *Lamarckiana*, although differing markedly in foliage. I unfortunately was unable to observe the early development of these plants and for this reason they were not very good forms on which to base studies of their progeny in the F_2 generation. Their seed was, however, sown this season and the cultures, described below, were of interest as indicating the probable behavior of hybrid plants of *biennis* and *grandiflora*. These four lines will not be cultivated further, since I have in the hybrids 10.30 La and 10.30 Lb material better suited to the purposes of a quantitative study for the reason that the records of their life history have been kept in detail. The seeds of these hybrids proved fertile to a very high degree, but it was practicable to grow only a small proportion of the seedlings to maturity.

1. Progeny from hybrid 9ba, *biennis* B \times *grandiflora*

D. This hybrid plant (Davis, '10, pp. 112 and 113), an excellent blend of the parent forms, was similar to *Lamarckiana* in habit and floral structure, but differed in having smaller, uncrinkled leaves on the lower portions of the plant and larger bracts upon the inflorescence.

From about 600 seedlings 73 plants were carried through the rosette stage and set in the ground, being selected to represent various types. The seedlings were strikingly diverse, some having long cotyledons similar to those of *grandiflora*, others having shorter and broader ones, and a large proportion with small light yellow, etiolated cotyledons. Many of the latter seedlings died before the appearance of the second leaf, the others developed very slowly, forming rosettes one-fourth or one-third the size of the normal with more or less etiolated leaves. Twenty-three of the dwarf rosettes were set out in the garden and of these seven finally grew to be large plants similar to the average of the culture, but with a somewhat etiolated foliage; of the remainder several died and the others developed into dwarf plants from 2-8 dm. high, small leaved, sparsely branched, and with flowers smaller than the average but larger than the *biennis* parent. The behavior of these etiolated dwarfs resembled De Vries's account of the appearance of the form *albida* in his cultures of *Lamarckiana*.

As the normal rosettes approached maturity it was possible to distinguish certain ones as somewhat more *biennis*-like or more *grandiflora*-like than the average, and the mature plants which developed from these showed similar points of resemblance to the respective parents of the cross. Nevertheless, the culture as a whole presented these parental characters well blended, although exhibiting a much wider range of variation than the F_1 generation of this cross. This variation appeared to indicate a relative segregation of the parental characters deserving of detail studies upon larger cultures. There were a number of plants similar to the parent hybrid, but none markedly nearer to *Lamarckiana*.

2. Progeny from hybrids 9ba, 9bb, and 9bc, *grandiflora* D \times *biennis* B. These three hybrids (Davis, '10, p. 114) presented the parental characters well blended. They were essentially similar to *Lamarckiana* in flower structure and inflorescence, but differed in foliage and habit, the leaves on the lower portion of the stem being but half the length of those similarly placed on *Lamarckiana* and with only slight traces of crinkles; the habit was *biennis*-like.

From about 350 seedlings of hybrid 9ba, 76 plants were brought to maturity, being selected as representative types of the rosette stages. A small proportion of the rosettes was dwarfed and the nine selected representatives of this type developed small plants 1-2.5 dm. high, generally without side branches; these did not flower. The normal rosettes varied greatly in the forms of leaves and extent of the red coloration, certain ones being distinctly more like the respective parents of the cross than the average; these differences were maintained in the mature plants, but to a less marked degree. The culture in general presented a habit more *grandiflora*-like than *biennis*-like, but all of the characters remained blended, although there was a considerable range of variation in flower structure and foliage. While a number of the plants were similar to the hybrid parents, none proved to be appreciably nearer to *Lamarckiana*.

There were about 550 seedlings of hybrid 9bb, from which 93 plants, selected as representative rosettes, were brought to maturity. Relatively few dwarf rosettes were present in this culture; eleven of these being selected grew into plants 1-4 dm. high, small leaved and without prominent side branches, the larger of the dwarfs developing small *biennis*-like flowers. The culture in general was more uniform than the preceding, but certain rosettes and mature plants were noticeably more like one or the other of the original parents than the average, which presented these parental characters well blended. The foliage of the culture was distinctly crinkled so that the plants re-

sembled *Lamarckiana* more closely than those of the other cultures in the F_2 generation, differing chiefly in the smaller size of the basal leaves and in the absence of red tinted papillate glands on a green stem, the stem being mottled with red. One plant of this culture (10.12 Lz) was selected for marked peculiarities, but will not be described unless its behavior in an F_3 generation proves of sufficient interest.

About 800 seedlings of hybrid 9bc appeared in the culture, from which 95 plants were selected as representative types of rosettes. A few dwarf rosettes were present, six of which set in the ground developed into unbranched plants about 1 dm. high, that failed to flower. The culture in general exhibited considerable variation, the most interesting types of plants being several with light green, smooth, obtusely pointed leaves, similar in shape to *Lamarckiana*, but without crinkles. There was shown the same previously described tendency on the part of a few rosettes and mature plants to depart from the average of the culture towards the characteristics of the respective parents of the cross, maintaining, however, a blended structure of their parts.

Considering these cultures of F_2 generations in comparison with the F_1 generations that have been grown, the most striking feature is the greater range of variation exhibited not only by the F_2 plants as a whole, but by their different parts. Since the studies were not quantitative in character, because such a large proportion of the seedlings were necessarily discarded, it has not seemed best to describe the variations in detail and such an investigation is deferred for the present. However, in this increased variation is clearly indicated at least a relative segregation of the parental characters in the F_2 generation.²

² Extensive cultures from the seed of the two hybrids 10.30 La and 10.30 Lb, described in this paper, are now (February, 1911) seedlings with 4-5 leaves which already show marked segregation in this F_2 generation, with the extreme types closely resembling seedlings of the parents of the cross and between these a large range of intermediates.

THE POSSIBLE ORIGIN OF *Ænothera Lamarckiana* AS A
HYBRID OF *O. biennis* AND *O. grandiflora*

We have shown that hybrids between certain strains of *Ænothera biennis* and *O. grandiflora* may be synthesized, which approach somewhat closely to *Ænothera Lamarckiana*, and there is good reason to believe that further experimentation will result in the production of forms with a more perfect resemblance. It is now important to ascertain, as far as this is possible, whether there are any historical reasons why *Lamarckiana* may not have arisen either accidentally or intentionally from such a cross.

Ænothera Lamarckiana appears to have been under cultivation in the gardens of the Muséum d'Histoire Naturelle at Paris in 1797, being described by Lamarck³ under the name *grandiflora*. Shortly afterwards Seringe⁴ renamed the form *Lamarckiana*, recognizing it to be distinct from the *grandiflora* of Aiton.

As previously noted from the investigations of MacDougal ('05) and Vail ('05), the evidence is very clear that *grandiflora* was introduced into England in 1778 and was at that time under cultivation at Kew. Forms of *Ænothera biennis* had of course been in European gardens for many years previous to 1778. There was therefore a period of about eighteen years (1778-1797) during which hybrids between *biennis* and *grandiflora* might have arisen in Europe before the earliest known record of the cultivation of *Ænothera Lamarckiana* in Paris. So striking an American novelty as *Ænothera grandiflora* would almost certainly have been passed on from Kew to other botanical gardens and in the interval between 1778 and 1797 is likely to have become widely distributed and cultivated. On historical grounds then there seems to be no reason with respect to the date of the first recorded recognition of *Ænothera Lamarckiana* why this form might not have arisen in Europe as a hybrid of *biennis* and *grandiflora*.

³ "Encyclopédie Méthodique Botanique," Vol. IV, p. 554, 1797.

⁴ De Candolle's "Prodomus," Vol. III, p. 47, 1828.

Let us suppose that it should be shown that *Oenothera Lamarckiana* was in existence previous to the date 1778, what effect would such evidence have on the hypothesis that the form is a hybrid of *biennis* and *grandiflora*? It would not in the writer's opinion have weight against experimental proof that *Lamarckiana* or forms closely resembling this plant may be synthesized as hybrids of these wild American species. It would not prove that Lamarck's plant in Paris (1797) was not a hybrid. It would merely indicate that *Lamarckiana*, having arisen as a hybrid in America, was introduced as such into Europe. As already pointed out, the position in *grandiflora* of the stigma well above the anthers gives ample opportunity for chance hybridization in nature. Indeed, the diverse forms that have appeared in my cultures from seed of *grandiflora* collected in the field clearly show that the species is far from homogeneous in character, a condition that is probably due to a large amount of cross pollination. It may be expected that careful search, especially in the southern United States, will bring to light occasional plants with characters intermediate between *grandiflora* and other species, such as, for example, southern types of *biennis*, but it is also probable that the behavior of such plants in culture will show them to be heterozygous in character, i. e., hybrids.

There have been two attempts to establish the presence of *Lamarckiana* in Europe previous to 1778 when *grandiflora* was introduced at Kew. MacDougal ('07, pp. 5, 6) refers to *Lamarckiana* a description and figure of an *Oenothera* by Miller, Plate 189, Fig. 2, for the "Gardener's Dictionary," 1760. This figure, published in 1757, is of a large-flowered *Oenothera* with petals 2 or 2.2 cm. long and by its side (Fig. 1) is a smaller-flowered form. With respect to the point under discussion, the most important features of these figures, clearly shown by the drawing, is the position and form of the stigmas, well below the tips of the anthers and with the lobes unexpanded in open flowers. These are peculiarities of

biennis and, in the writer's experience, are not characteristic of *Lamarckiana* where the stigma lobes are usually expanded in the open flower and generally above or about on the level with the tips of the anthers. Furthermore the size of the petals in the illustration of the large-flowered type (Fig. 2) is no greater and indeed not so great as in some forms of *biennis*. Both of the figures show the essential characteristics of the flower of *biennis* to which they have generally been referred in taxonomic accounts. For these reasons the view of MacDougal that the illustration of the large-flowered type (Fig. 2) is of *Lamarckiana* and establishes its presence in Europe previous to 1757, is to the writer not convincing.

The second attempt to establish the presence of *Lamarckiana* in Europe previous to 1778 is the announcement of Gates ('10) that certain marginal notes in a copy of Bauhin's "Pinax," 1623, give in Latin an accurate description of this plant although differing in one or two minor characters. Gates presents an outline of the points which indicate to him that the description refers to *Lamarckiana*, but the notes themselves are not published. A full account is promised, in which we may expect to see these Latin notes and judge of them for ourselves, and comments on this announcement will be reserved for the present.

Finally we must return to the question of whether or not it appears probable that *Oenothera Lamarckiana* is at present a component of the American native flora. De Vries ('05, p. 368) refers to *Lamarckiana* certain herbarium material at the New York Botanical Garden and Missouri Botanical Garden, both collected by A. W. Chapman in Florida (1860 or earlier), and also material in the Philadelphia Academy of Science collected by C. W. Short at Lexington, Ky. A thorough search (MacDougal, '05, p. 6) by several botanists in the vicinity of Lexington Ky., Nashville Tenn., Knoxville Tenn., and Courtney Mo., in the endeavor to find living plants that might be identified as *Lamarckiana*, was unsuccessful.

ful. Later, Miss Vail (MacDougal, '07, p. 67) came to the conclusion that the plant from Lexington, Ky., is *grandiflora*, and a possible escape from cultivation. I have not seen the herbarium material mentioned above, but in the light of the fact that many dried specimens could be prepared from my hybrids which as such would be considered *Lamarckiana*, it is clearly necessary that evidence from herbarium material should be weighed with much caution. The average herbarium material of the *Oenotheras* is generally not sufficient to show the peculiarities of the earlier phases of development (rosettes and basal foliage) which in the case of *Lamarckiana* furnish diagnostic characters that are necessary for a full identification. Unless the evidence of field collections is followed up by garden cultures, there is the possibility of numerous errors of interpretation.

A specimen in the Gray Herbarium of Harvard University is stated by MacDougal ('05, p. 5) to agree perfectly with *Oenothera Lamarckiana*, but in this view the writer can not accord. This plant was apparently grown in the Cambridge Botanical Garden, Massachusetts and bears the date 1862. The specimens are accompanied by the significant notes in the hand writing of Dr. Asa Gray "from seed of Thompson, Ipswich," and "said by English horticulturists to come from Texas." The flowers are large, with petals about 4.5 cm. long and sepals about 5 cm. long, very attenuate, the tips projecting 1 cm. beyond the folded petals in the manner characteristic of *grandiflora*. The stigma lobes are also *grandiflora*-like in their length, about 8 mm., and in their position, about 5 mm. above the tips of the anthers. A large detached leaf, about 18.5 cm. long, with some evidence of former crinkles, suggests by its form (although rather small) the basal leaves of *Lamarckiana*. The flowers and upper foliage of this specimen, however, agree very closely with broad-leaved types in my cultures of *grandiflora* and do not resemble the *Lamarckiana* that I have grown from seeds of De Vries, or with his figures and descriptions in "Die Mutations-

theorie." If this plant could be established as derived from *Oenotheras* introduced into England by Messrs. Carter and Co. at about 1860 from seeds said to come from Texas, it would be a point of great importance, as will appear in the following paragraphs.

De Vries ('05, pp. 384-385) offers strong evidence that the strains of *Lamarckiana* at present cultivated in Europe have a genetic relation to seed of Messrs. Carter and Co., of London about 1860. This seed is stated to have been received unnamed from Texas and plants grown from it were pronounced by Dr. Lindley to be *Lamarckiana*. A specimen from one of these plants is figured in "The Floral Magazine," Vol. II, Plate 78, 1862, this plate being reproduced in "L'Illustration Horticole," Vol. IX, Plate 318, 1862. This plate shows an *Oenothera* with flowers about 10 cm. (4 inches) in diameter and with a large amount of red coloration on the sepals and ovaries; the stigma is figured both above and below the tips of the anthers. The flowers of this illustration are larger than those of *Lamarckiana*, as known to the writer, and would do for *grandiflora* except for the position of the stigma which is much closer to the anthers than is typical for this species. The red coloration of the sepals and ovaries is much too deep for typical *Lamarckiana* and not unlike some forms of *grandiflora*, but the sepal tips, as drawn, are not so long or so pointed as in the latter form. Indeed the identification of this plate with any probable *Oenothera* is very difficult and the reasons why it should be called *Lamarckiana* are to the writer far from convincing, although it would perhaps be as easy to argue for this identification as for any other.

It is, however, possible that new light may be thrown on the composition of the cultures of Carter and Co. through the plant in the Gray Herbarium described above. The date of this specimen, 1862, together with the very suggestive notes of Dr. Gray "from seed of Thompson, Ipswich," and "said by English horticult-

tourists to come from Texas," make it appear possible that this plant was derived from the cultures of Carter and Co. If this could be established it would indicate that forms very close to *grandiflora* were present in the cultures or seeds of this firm. It is not at all improbable that Texas with its immense area and very great range of climatic conditions may harbor *grandiflora* or related types especially since it is known to be rich in species of *Oenothera* and to have a number of large flowered representatives.

There may have been thus a second introduction into England of *grandiflora*-like types through Carter and Co. at about the year 1860. While there is of course no means of knowing whether their cultures were uniform, it is altogether probable that the results of their sowings gave a diverse progeny, since that has been my experience with seed from Alabama. There seems to be no reason why chance hybrids may not have been present or why *grandiflora*-like strains might not have shortly hybridized with European forms of *biennis*. These possibilities are mere matters of speculation to which little assistance is given by the puzzling plate in "The Floral Magazine" and in "L'Illustration Horticole" referred to above. A search among the English herbaria might, however, result in the discovery of specimens which would materially assist in the solution of a very interesting question—the identity of the plants grown by Carter and Co. At present the specimen in the Gray Herbarium appears to offer the most important evidence bearing upon the question. The contention that *Lamarckiana* was introduced in the form of a native American species at this date, 1860, seems to the writer to be without sufficient foundation.

The American botanist will ask himself why, if *Lamarckiana* was present in America as a native species in 1860, no localities are known where it may be observed in the field. It will be hard for him to believe that so strong and vigorous a plant, if a wild species, has become

so recently extinct when, as he well knows, the *Ænotheras* are established as remarkably successful forms in our flora. The fact that *Lamarckiana* is not known as a component of the native American flora stands as the most serious obstacle to the view that this plant is representative of a wild species. The writer believes it very probable that plants more or less resembling *Lamarckiana* will occasionally, or perhaps rarely, be found in parts of America and under circumstances indicating that they are not garden escapes, but it seems to him equally probable that these forms when tested in culture will give evidence of a heterozygous, or hybrid nature. The mere records of such plants as handed down by the average type of herbarium specimen, unaccompanied by experimental cultures, will have little or no value for the present problem—the origin of *Ænothera Lamarckiana*.

SUMMARY

This paper offers a body of evidence which shows that hybrids resembling *Ænothera Lamarckiana* may be synthesized from certain strains of the American native species *O. biennis* and *O. grandiflora*. The resemblances of the hybrids to this plant are strongest with respect to the inflorescence, buds and flowers. The differences are chiefly manifest in the basal foliage of the mature plant, in the coloration of the stem, and in the more straggling habit of the hybrids. The rosettes of the hybrids present mixed forms of leaves, the younger with points of similarity to *Lamarckiana*. Bearing in mind that other strains of *biennis* have characteristics more *Lamarckiana*-like than those of strains A and B, herein described, it is more than probable that the hybrids from certain crosses made this season (1910), when grown in future cultures, will come closer to the desired end—the synthesis of a hybrid so similar to *Lamarckiana* as to be practically indistinguishable by the usual taxonomic tests.

Exception is taken to the claim of MacDougal ('07,

pp. 5, 6) that Miller's Plate 189, Fig. 2, for the "Gardener's Dictionary," 1760, establishes the presence of *Lamarckiana* in Europe previous to the date, 1778, when *grandiflora* is known to have been introduced into England. The view of De Vries that strains of *Lamarckiana* were introduced into England about 1860, through seed of Messrs. Carter and Co. said to come from Texas, is discussed with reference to certain specimens in the Gray Herbarium of Harvard University and in the light of the author's experience with seed from Alabama, indicating that Carter and Co. probably had *grandiflora*-like types in their cultures which were also likely to have been of a mixed character. The absence, so far as is known, of *Lamarckiana* as a component of the native American flora is emphasized as a point of great importance against the claim that *Lamarckiana* was introduced into Europe as an American wild species.

A working hypothesis is presented as a result of the writer's experimental studies and in relation to such historical evidence as is available, to the effect that *Oenothera Lamarckiana* arose as a hybrid between certain types of *biennis* and *grandiflora*, recognizing that under these names must, for the present at least, be included a number of races which can only be clearly defined by laborious genetical investigations. The precise time and place of such an origin for *Lamarckiana* is a matter of mere speculation, but there seems to the writer no good reason why hybridization between *biennis* and *grandiflora* might not have taken place in Europe between 1778 and 1797 (when *Lamarckiana* was first recognized at Paris) and also at later dates, as for example about 1860. It is also possible that *Lamarckiana* may have been introduced as a chance hybrid from America, but the probability of such an origin is naturally rather remote.

The bearing of the possible hybrid nature of *Oenothera Lamarckiana* upon the claim of De Vries that the behavior of this plant demonstrates the origin of new species

by mutation from a form representative of a typical wild species will be sufficiently evident to require no comment at this time. A discussion of the matter will therefore be reserved until the writer has proceeded further with his studies.

CAMBRIDGE, MASS.,
November, 1910.

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THE GENOTYPES OF MAIZE¹

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THE doctrine of evolution had to overthrow the conception of permanency of specific types, generally held when Darwin's "Origin of Species" was published, because that conception was then associated with the idea of a separate original supernatural creation of each such type. It was Darwin's great triumph that he succeeded in marshaling such an array of facts pertaining to variability, as to convince the scientific world—and through the scientific world, ultimately the whole world—that everything is in a state of flux, and that there is no such thing as permanency among living things.

Owing to the work of De Vries and the other early students of modern genetics, permanency of type again demands serious scientific consideration, for such permanency is no longer incompatible with the doctrine of evolution, being now associated with some form of the mutation theory. The old idea of the immutability of specific types was based upon almost total ignorance of genetics, as was likewise the Darwinian conception of fluidity and gradual change, for although many appeals were made by Darwin to the experiences of plant and animal breeders, it is now known that these experiences were the result of no such careful control of conditions or analysis of results as has been found necessary for the discovery of genetic laws. The critical work of the past few years has wrought a great change and the new idea of permanency is gaining ground with the growth of experimental knowledge.

Without granting that we have yet reached a position in which we can say definitely that types are absolutely

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permanent and do not, at least in some cases, gradually change into something new, the large accumulation of

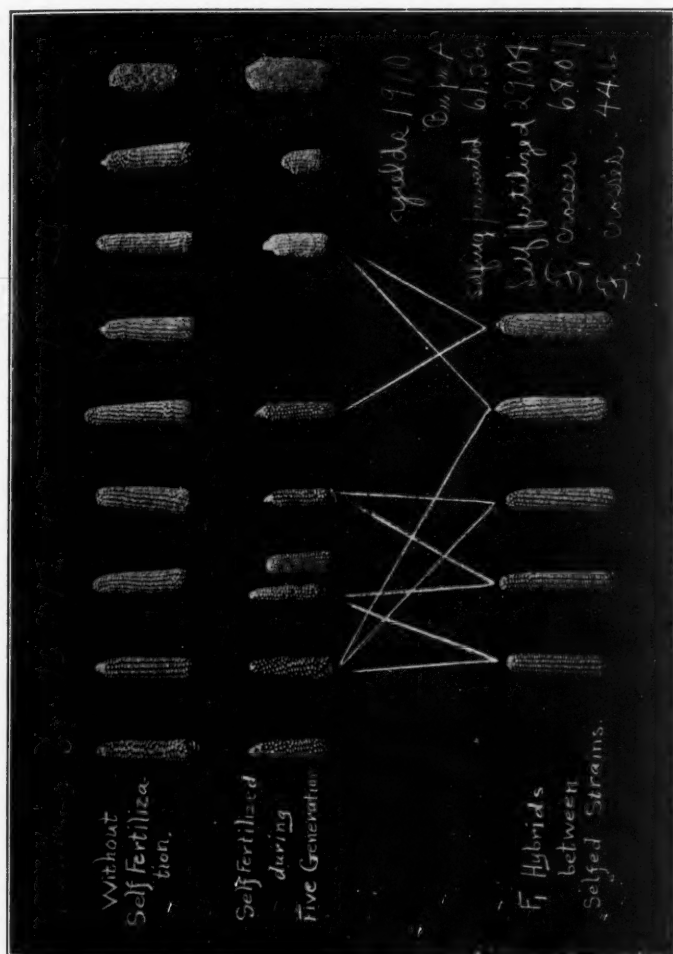


FIG. 1. Each ear in this exhibit represents a different pedigreed family. In each family the variation was slight, and the ear chosen for the exhibit was fairly representative of the entire family. The two self-fertilized ears under selection number "12" belong to two distinct strains, the left-hand one being my "Strain A" of other publications. During the last two years this has been selected to its own modal number, while the right-hand ear has been selected to twelve rows throughout the course of the experiments.

experimental data now available makes it necessary to recognize a clear distinction between the evolutionary changes in types, on the one hand, and the fluctuations within each type, on the other hand.

Quite naturally the first experimental evidence of the existence of permanent hereditary types involved only such characteristics as are clearly distinguishable upon inspection. Thus Jordan was able to demonstrate that within the systematic species *Draba verna* there are included as many as two-hundred hereditary forms, whose distinguishing characteristics appear unchanged from generation to generation, in such manner that his pedigrees of these forms were clearly and permanently distinguishable from each other by easily defined morphological features. Such "*petites espèces*" or "little species" (afterwards called by De Vries "elementary species," and by Johannsen "biotypes" or "genotypes"), have since been observed by Wittrock and his students, and by many others, in a great number of wild species, and they are now quite generally supposed to be of almost universal occurrence.

About 1890 N. H. Nilsson made a similar discovery in connection with his breeding of wheat, oats, barley and other grains at Svalöf, Sweden, but his work remained practically unknown to the scientific world until it was brought to light several years ago by De Vries. Nilsson found in these grains elementary species, each with its own morphological characters and its own specific capacity to yield crops of given size or quality under given external conditions. More recently, sharp-eyed taxonomists have been rapidly raising many of the elementary species of wild plants to the rank of systematic species.

It was natural that the earliest genotypes recognized, such as those of Jordan and Nilsson, should have possessed visibly discrete characteristics, and that they should first have become familiar in normally self-fertilized plants, among which little confusion is occasioned

by the rare crossing of unlike individuals. Great credit is due to Johannsen² for demonstrating that in such self-fertilized plants, types also exist which are not readily distinguishable by simple inspection, but whose occurrence may be completely demonstrated by the refined methods of the mathematician. Not only has Johannsen's work been so extensive as to justify the conclusions arrived at by him, but various other investigators, working with different classes of research material, have shown that the conditions found by Johannsen in beans and barley are duplicated in many other species and varieties. Perhaps the strongest support in this direction has come from the work of East³ with potatoes and that of Jennings⁴ with various microscopic organisms, especially with paramecium.

The fact that *Draba verna*, and many other wild species in which the existence of numerous elementary species has been demonstrated, as well as wheat, oats, barley and beans, are all predominantly self-fertilizing, and that potatoes and paramecium have an asexual reproduction, has led some to the erroneous notion that the discreteness, uniformity and permanence of the types which have been discovered among these and other similar organisms, are in some way dependent upon the absence of crossing.

It must be admitted that conclusions drawn from self-fertilized and asexual material do not necessarily apply to plants and animals whose successful existence is dependent upon repeated crossing. Nevertheless, the conception of pure and permanent genotypes in cross-bred material has become familiar simultaneously, owing to the work done in Mendelian heredity; for homozygous

²Johannsen, W., "Ueber Erbllichkeit in Populationen und in reinen Linien," 68 pp., Jena, 1903.

³East, E. M., "The transmission of variations in the potato in asexual reproduction," Conn. Exp. Sta. Report 1909-1910, pp. 119-160, 5 pls.

⁴Jennings, H. S., "Heredity, variation and evolution in Protozoa—II. Heredity and variation of size and form in Paramecium, with studies of growth, environmental action and selection," *Proc. Amer. Phil. Soc.*, 47: 393-546, 1908.

combinations of the various characteristics of plants and animals "breed true" to those characteristics. Just as the first recognition of permanent differences in pure lines involved easily distinguishable characters, so also these first discoveries of permanent pure-breeding genotypes in cross-bred plants and animals involved easily definable morphological characteristics. The demonstration that in normally pure-bred lines there are distinctions more minute than such easily visible features as characterize the elementary forms of *Draba* and many other species, was an important advance in our analysis of the populations which make up the species of plants and animals. A similar demonstration that populations of cross-breeding plants and animals are composed of fundamentally distinct types, intermingled but not changed by panmixia, and capable of being separated by appropriate means and of being shown to possess the discreteness, uniformity and permanence already demonstrated for the genotypes of self-fertilized and clonal races, will add greatly to the importance of the fundamental conception of permanency of types involved in the work of De Vries and Johannsen.

For the study of this problem there is probably no better plant than Indian corn. It is known to exist in a large number of obviously distinct strains or subspecies which cross together with the greatest ease. Many of its characteristics have been proved by different investigators to be Mendelian unit-characters; such, for instance, as the color of the seed-coat, whether red, dark yellow, light yellow, variegated or colorless, the color of the aleurone layer, whether blue, red or white; the color of the endosperm, whether yellow or white; the chemical composition of the endosperm, whether starchy or sugary, the color of the silks and cobs whether red or white, etc. It has become known also, mainly through the excellent work done at the Illinois State Experiment Station, that oil-content and protein-content of the grains, the position of the ears, the number of ears on the stalk, and

several other characters, are capable of accentuation by selection, so that different degrees of these qualities are capable of being made characteristics of particular strains of corn, without there being the least evidence as yet that these last-mentioned qualities bear any relation to the unit-characters with which the student of genetics generally deals. A further point in favor of maize as a subject for the study of genotypes among cross-breeding organisms lies in the fact that its flowers are so arranged that, while self-fertilization is possible, it is naturally almost completely excluded, thus ensuring the same relations as are presented by bi-sexual or diœcious plants and animals, while retaining the means of conveniently testing the genotypic nature of each individual by controlled self-fertilizations.

I think I have demonstrated during the last five years that there are many genotypes of Indian corn which, although they can not always be distinguished by definable external characteristics, can be proved to be just as certainly and permanently discrete as the types whose distinguishing features can be recognized as Mendelian unit-characters. I shall endeavor to show, in what follows, a portion of the evidence which leads me to this conclusion.

In 1905 I undertook a rather extensive series of comparisons between cross-bred and self-fertilized strains of Indian corn for the purpose of discovering the effects of these methods of breeding upon variability, and these investigations have been continued each year since that time. Two phenomena immediately attracted my attention: First, the well-known fact that the children of self-fertilized parents are inferior to those of cross-fertilized parents in height, yield and other characters dependent in any way upon physiological vigor. In every instance this phenomenon was plainly evident in the very first generation after self-fertilization. This decrease in physiological vigor due to self-fertilization has become an

extremely important relation in the study of the genotypes, as will be shown later.

The second phenomenon which quickly made itself manifest, was first clearly appreciated in the second generation after the beginning of the experiments; this was the fact that each self-fertilized family possessed morphological features which clearly differentiated it from all other families. In most cases the distinguishing characteristics of these families were of such elusive nature that it was impossible to recognize definite unit-characters, and indeed, morphological descriptions of the several pedigrees could often be made only in terms of greater or less intensity of the several qualities exhibited. However, the distinctions were real and applied to every member of the particular family. Thus one family might have a very slender, poorly developed male panicle, while another would have more thick and dense branches of the panicle. This difference might be quite small when given in actual measurement but inspection showed that every individual of the one family had the slender, illy developed panicles, while all of the offspring of the other family had the thicker, denser type. Similarly, one family might have a slightly broader and darker green leaf than another, and these characteristic differences were likewise uniformly present in all members of the single families contrasted. No such character as this is capable of being traced through the generations following a cross, in the manner usually pursued by the geneticist, and the matter must be approached by indirect methods. The important point to be kept in mind here is simply that *the self-fertilized families, derived originally from a common stock, do differ by morphological characteristics*, and that there comes to be great uniformity in regard to the presence of these characteristics in all the individuals of a given self-fertilized family.

This relative uniformity, which is so obvious even to the casual observer, is not sufficient in itself, however,

to positively demonstrate the existence of distinct genotypes in maize, because the slight variations which must always be present even in the most uniform progeny, can not be certainly distinguished as genotypic or fluctuating simply by inspection. Such demonstration must rest upon a combination of biometric and genetic evidence in order to prove acceptable. Most of the differentiating characters of my several strains of maize are such that they do not lend themselves readily to biometric methods, but the number of rows on the ear is well adapted for such study and several important results have been derived from the consideration of this character. An important proof that the self-fertilized families derived from my common original stock of corn are genotypically distinct, and that they do not owe their different morphological and physiological qualities to fluctuations within a single genotype, was quickly found in the fact that two of these families selected respectively to 12 and 14 rows of grains on the ears, showed a regression of row-number toward different centers instead of toward a common center. The mean of the original population was slightly above 14 rows. The selection to 14 rows was very near this mean and the selection to 12 rows was very near this mean and the selection to 12 rows considerably below it. According to Galton's well-known law of "regression toward mediocrity," the mean of a family whose parents were selected to 12 rows should have lain somewhat above 12 rows, and that selected to 14 rows should have retained the mean approximately at 14 rows. The actual result in the case of selection to 12 rows was the production of a family having a mean row-number considerably below the number of rows selected, and the subsequent generations have since shown a close approach to an 8-rowed condition; while the family whose parents were in each generation selected to 14 rows has always had the mean very near to 14 rows. As these families were grown under as nearly uniform conditions as possible, the fact that the 14-rowed family continues

to have its mean row-number at 14 shows that the fall in row-number from 12 to 8 in the other family has been due to internal rather than to external causes.

The change in variability in number of rows on the ears has also been studied from year to year. Continued self-fertilization has resulted in a gradual decrease of variability in the number of rows per ear in each of the self-fertilized lines. This is a fluctuating character, and so far as present evidence goes, the number of rows per ear in any strain can not be fixed at a definite number. While it is probable that none of my self-fertilized families has yet reached an absolutely pure-bred condition, several of them have become so nearly pure-bred that their various relations can be used to demonstrate that they are approaching purity as a limit.

In 1909 two of these nearly pure-bred families were compared with their reciprocal hybrids in the first and second generations, with reference to the variability in number of rows.⁵ It was found that the average variability in these two self-fertilized families was 9.08 per cent. The variation in number of rows in their F_1 progeny was 9.06 per cent., and in the F_2 12.63 per cent. A comparison of these coefficients of variability shows at once that the variation in number of rows in the F_1 is essentially identical with that in the self-fertilized lines used for the cross. Theoretically this should be so if the strains used were pure genotypes, because in that case all germ-cells in each pure strain were alike, and therefore, when individuals belonging to these two lines were crossed, equal sperms met equal eggs; consequently there should be no variability in their offspring due to germinal differences, but only those due to environment in the widest sense. As the pure-bred families and their F_1 and F_2 progenies were grown beside each other during the same season, they were subjected to as nearly identical environmental influences as can be attained.

⁵ Shull, G. H., "Hybridization methods in corn breeding," *Am. Breeders' Magazine*, 1: 98-107, 1910.

Consequently, when the F_1 shows the same variability as the pure lines which entered into it we must conclude that there was at least approximate equality among the sperms which came from the one self-fertilized strain, and among the eggs which came from the other. In the F_2 , on the other hand, genotypic differences appear, owing to the segregation of the different characteristics into the different germ-cells, and to this fact may be ascribed the increased variability in the F_2 .

While other characters have not been studied by the same methods that have been used in the investigation of the number of rows on the ears, several features associated with the physiological vigor of the various pedigrees have given evidence which appears to me to be strongly corroboratory of the uniformity of the germ-cells produced by plants which have become pure-bred through continued self-fertilization. The smaller size and less vigor of the offspring of self-fertilized plants as compared with those from a normally cross-bred plant were formerly taken to indicate that self-fertilization is injurious, and Darwin's "Effects of Cross and Self-fertilization in the Vegetable Kingdom" strongly impressed this point of view. I have been able to demonstrate, however, that this supposedly injurious effect of self-fertilization is only apparent and not real; or at least that if there is such injurious effect, it is relatively insignificant as compared with the increased vigor due to heterozygosis. The most important evidence of this is found in the fact that the continuation of self-fertilization in any pedigree does not produce a corresponding decrease in vitality and size. The decrease resulting from a second year of self-fertilization is not as great as that from the first year. The third year of self-fertilization produces still less deterioration, and as this process is continued a limit is approached in such manner as to justify the inference that when complete purity is attained no further deterioration is to be expected, thus proving that self-fertilization is not in itself injurious.

That this is also true of other plants is derivable from Darwin's own work.

This decrease in size and vigor is accompanied by the gradual lessening of variability, and when that state is finally reached in which there is no further decrease in size and vigor, it seems probable that there will be also no further noticeable change in variability. This does not mean, of course, that there will be no variability, for even the most uniform group of plants or animals will of necessity show slight variations produced by different conditions of life, food supply and so forth. But present evidence does not warrant the belief that such fluctuations affect in the least the fundamental qualities of the genotype.

In 1908 I suggested a hypothesis to explain the apparent deterioration attendant upon self-fertilization, by pointing out that in plants, such as maize, which show superiority as a result of cross-fertilization, this superiority is of the same nature as that so generally met with in F_1 hybrids. I assumed that the vigor in such cases is due to the presence of heterozygous elements in the hybrids, and that the degree of vigor is correlated with the number of characters in respect to which the hybrids are heterozygous. I do not believe that this correlation is perfect, of course, but approximate, as it is readily conceivable that even though the general principle should be correct, heterozygosis in some elements may be without effect upon vigor, or even depressing. The presence of unpaired genes, or the presence of unlike or unequal paired genes, was assumed to produce the greater functional activity upon which larger size and greater efficiency depend. This idea has been elaborated by Dr. East⁶ and shown to agree with his own extensive experiments in self-fertilizing and crossing maize. He suggests that this stimulation due to hybridity may be analogous to that of ionization.

Mr. A. B. Bruce proposes a slightly different hypothe-

⁶ East, E. M., "The distinction between development and heredity in in-breeding," *AMER. NAT.*, 43: 173-181, 1909.

sis in which the degree of vigor is assumed to depend upon the number of *dominant* elements present rather than the number of *heterozygous* elements. While all of my data thus far are in perfect accord with my own hypothesis, and I know of no instance in which self-fertilization of a corn-plant of maximum vigor has not resulted in a less vigorous progeny, it is quite possible that I have still insufficient data from which to distinguish between the results expected under these two hypotheses. However, for the purpose of the present discussion, it is not necessary to decide which of these two hypotheses (if either) is correct. Both of them are based upon the view that the germ-cells produced by any plant whose vigor has been increased by crossing are not uniform, some possessing positive elements or genes not possessed by others.

Several different characters which are more or less dependent upon physiological vigor have been taken into account in my work, each of which gives its own support to the conception upon which both of these hypotheses are based. The number of rows of grains on the ears which has been most extensively used as a measure of variability, and as a guide in selection, is found to be somewhat affected by the vigor of the individual, and it is due to this fact, no doubt, that the row-number is a fluctuating character, even in the pure genotype. Another characteristic which has been used as a measure of vigor has been the yield of corn computed in bushels per acre.⁷ A third characteristic, which was not taken into account at the beginning of the experiments but which has given confirmatory data in the later years, is the height of the stalks, a character which was much used by Darwin as a measure of vigor in his study of the effects of cross- and self-fertilization in plants.

⁷ It should be understood that this method of stating yields is seriously defective, in that it implies the existence of a much smaller probable error than is actually present, since each of my pedigrees has usually occupied only about one one-hundredth of an acre. However, I believe that this defect is more than offset by the advantage of using a unit of yield with which all readers are familiar.

We may now consider the behavior of these several measures of physiological vigor in relation to the theory that distinct genotypes of maize are gradually segregated from their hybrid combinations, by self-fertilization, and that the degree of vigor is correlated with the degree of heterozygosis.

I have kept families selected to given numbers of rows on the ears—one series of families repeatedly self-fertilized and another series repeatedly crossed with mixed pollen in such a manner that self-fertilization is precluded by artificial means. It is not practicable to do this crossing with mixed pollen in such a manner as to duplicate the conditions found in an ordinary corn-field for the simple reason that the number of individuals which contribute the pollen must be more greatly restricted than is true in the open field. While self-fertilization has been entirely prevented, there has been a degree of in-breeding somewhat greater therefore than will occur under non-experimental conditions. This degree of in-breeding is sufficient to slowly eliminate some of the hybrid elements which were originally in my strain of corn and should consequently lead to a gradual deterioration in case my theory of the relation between vigor and hybridity is correct. As a matter of fact, such deterioration has become apparent in the "cross-bred"⁸ families, when measured either by height of stalk or yield per acre, though both of these measures show that the deterioration has been slight. It is so slight, indeed, that it is very much exceeded by the fluctuations from season to season, and may only be demonstrated by the application of a correction which approximately eliminates this seasonal fluctuation. When we compare this continual slight fall in physiological vigor of the cross-

⁸It should be noted that here and in what follows I use the expression "cross-bred" in a special sense, to denote the fact that all self-fertilization has been avoided. The more usual use of the term "cross-bred" to denote a cross between individuals belonging to distinct strains, I replace in this paper by the expression "F₁," as I can see no tangible distinction between such a cross, and hybridization in the older, more restricted, and more arbitrary sense.

bred families with the changes produced in the self-fertilized families during the same period, there is a striking contrast, for in the latter case there was great decrease in height and yield in the first year, a considerably less decrease in the second year of self-fertilization, still less in the third year, and so on, and while I have evidence that none of my self-fertilized families has yet reached a state of perfect stability, they are at the present time decreasing in regard to both of these measures of vigor somewhat less rapidly under continued self-fertilization than are the families in which self-fertilization has been absolutely precluded.

Necessary corollaries of the view that the degree of vigor is dependent on the degree of hybridity, or, in other words, that it is dependent roughly upon the number of heterozygous elements present and not upon any injurious effect of in-breeding *per se*, are (a) that when two plants in the same self-fertilized family, or within the same genotype, however distantly the chosen individuals may be related, are bred together, there shall be no increase of vigor over that shown by self-fertilized plants in the same genotype, since no new hereditary element is introduced by such a cross; (b) that first generation hybrids produced by crossing individuals belonging to two self-fertilized lines, or pure genotypes, will show the highest degree of vigor possible in progenies representing combinations of those two genotypes, because in the first generation every individual will be heterozygous with respect to all of the characters which differentiate the two genotypes to which the chosen parents belong, while in subsequent generations, recombination of these characters will decrease the average number of heterozygous genes present in each individual; (c) that crosses between sibs among the first-generation hybrids between two genotypes will yield progenies having the same characteristics, the same vigor, and the same degree of heterogeneity, as will be shown by the progenies of self-fertilized plants belonging to the same first-generation family.

All of these propositions have now been tested in a limited way. In 1910 nine different self-fertilized families were compared with nine crosses between sibs within the same self-fertilized family; ten crosses between sibs in F_1 families were compared with ten self-fertilizations in the same F_1 families; seven families were raised as first generation hybrids between individuals belonging to different self-fertilized families; and ten families were grown, in which self-fertilization had been entirely precluded during the past five years. The average height of plants in decimeters, the average number of rows per ear, and the average yield in bushels per acre, in these fifty-five families are given in the following table:

	Selfed \times Self	Selfed \times Sibs	F_1	F_2	$F_1 \times$ Self	$F_1 \times$ Sibs	Cross- breds
Av. Height	19.28	20.00	25.00	23.42	23.55	23.30	22.95
Av. Rows	12.28	13.26	14.41	13.67	13.615	13.73	15.13
Av. Yield	29.04	30.17	68.07	44.62	41.77	47.465	61.52

An examination of this table indicates to me that on the whole my self-fertilized families are not yet quite pure-bred; for the sib crosses give on the average a slightly greater height, number of rows per ear, and yield per acre than the corresponding self-fertilized families, as shown by a comparison of the first two columns of the table. The same fact is apparent from a comparison of the " $F_1 \times$ self" and " $F_1 \times$ Sibs" columns, except that in this case the heights and number of rows per ear are essentially equal while the yield per acre is significantly higher in the sib-crosses than in the self-fertilized families. An alternative explanation of these slight differences between the results of self-fertilization and of sib-crosses may attribute them to an injurious effect of self-fertilization, but in any event such injurious effect must be exceedingly slight as compared with the stimulating effect of heterozygosis. My practise of choosing for seed the best available ears tends to delay the attainment of complete genotypic purity, and this fact favors the view that whatever advantages the sib-

crosses show, are attributable to this lack of purity, rather than to any advantage gained by crossing *per se*.

The columns of the table representing the F_1 and F_2 show very plainly the superiority of the former over the latter in regard to both height and yield per acre. The fall in average height from F_1 to F_2 from 25 decimeters to 23.4 decimeters and the corresponding fall in yield per acre from 68.07 bushels in the F_1 to 44.62 bushels per acre in the F_2 show in a most striking way the economic advantage of using first-generation hybrids for producing the corn crop. A comparison of the F_1 hybrids with the "cross-breds" shows the average yield of the former to be 6.55 bushels per acre greater than that in the families in which self-fertilization had been avoided.

The relation of these results to the experiences of economic breeders of corn may now be considered. Perhaps in no other class of plants has the evidence been so strong for the possibility of gradual improvement through continued selection as in corn, and this method has been generally followed. The selections of particular physical and chemical qualities which have been carried on at various experiment stations have produced noteworthy results. Most important instances of this kind are involved in the breeding experiences of Hopkins, Smith and other breeders at the Illinois State Experiment Station, which have been already mentioned. Here selections for high oil content, low oil content, high protein and low protein, high ears and low ears, and the angle which the ears make with the axis of the plant, as well as selection for increased yields, have all led to the production of strains which possessed the desired qualities to a much higher degree than that in which they existed in the foundation stock when the selection began. All of these results may be readily explained on the ground that some hybrid combinations of genotypes have greater capacity for the production of the desired qualities than other combinations, and that the selection has gradually brought about the segregation of those genotype-combinations

which had the highest capacity for the production of the desired qualities. At least in regard to yield and not improbably also in regard to the other qualities for which selections were made, the results were dependent, not upon the isolation of pure types possessing the desired quality, but upon the securing and maintaining the proper combination of types. I have shown above that segregation takes place in a manner at least similar to, if not identical with, the well-known behavior of Mendelian characters. As a consequence of this, no strain of corn can be maintained at a high value with respect to any quality whose development is correlated with heterozygosis, except by continued selection for the particular qualities desired. If in any such specialized strain selections should be made for a few years on the basis of some character independent of the one used in establishing the strain, the superior qualities for which it was originally selected would quickly disappear, owing to the breaking up of the efficient combinations which had been segregated and maintained by selection.

The principles here presented have very great potential consequence for the practical grower of corn, and possibly for the breeder of many other cross-breeding plants and of animals. Their importance seems not to have been fully appreciated by any one however, until recently, though several breeders appear to have glimpsed the possibilities at one time or another. Thus G. N. Collins,⁹ of the United States Department of Agriculture, has recently shown that several breeders at different times began experiments to test the value of hybridization in the production of high-yielding strains of corn. The first attempt of this kind which he has found was that of W. J. Beal¹⁰ at the Michigan Agricultural College in 1876. At Professor Beal's instance several other experiment stations undertook to work in co-operation with the Michigan Station in testing the value of hybrids in

⁹ Collins, G. N., "The value of first generation hybrids in corn," Bull. 191, U. S. Bureau of Plant Industry, 45 pp., 1910.

¹⁰ Beal, W. J., Reports, Michigan Board of Agriculture, 1876-1881.

corn breeding, but only Professor Ingersoll,¹¹ of Purdue University, reported results. Professor Sanborn¹² apparently performed similar experiments in the late eighties at the Maine Agricultural Experiment Station. In 1892 G. W. McCluer¹³ reported on a number of crosses made during the preceding two years at the Illinois Agricultural Experiment Station, and during the next two years Morrow and Gardner¹⁴ published bulletins from the same station, describing the results of a number of crosses. Apparently none of this work led to the subsequent utilization of hybridization methods in corn breeding, as no work along this line appears to have been done between the time when Morrow and Gardner issued their second bulletin in 1893 and the publication of the first report of my work with corn at the Station for Experimental Evolution in 1908. The work of Beal, Ingersoll, Sanborn, McCluer, and Morrow and Gardner showed that increased yields from the hybrids, as compared with the strains used for the crosses, are the almost invariable result, though both McCluer, and Morrow and Gardner found isolated instances in which the hybrids were inferior to the parent strains. Hartley¹⁵ has since reported that among a number of crosses made by the United States Department of Agriculture also, some gave poorer yields than the parent strains used for the cross, while others gave superior yields, and reached the conclusion, which I think is justified by my own results, that promiscuous crossing is not necessarily advantageous but that certain combinations lead to increased yields while others may prove disadvantageous. Collins¹⁶ has

¹¹ Seventh Annual Report of Purdue University, 1881, p. 87.

¹² Sanborn, J. W., "Indian corn," Agriculture of Maine, 33d Annual Report, Maine Board of Agriculture, 1889-90, p. 78.

¹³ McCluer, G. W., "Corn crossing," Bull. 21, Illinois Agr. Exp. Sta., 1892, p. 85.

¹⁴ Morrow, G. E., and Gardner, F. D., Bulletin 25, pp. 179-180, and Bulletin 31, pp. 359-360, Illinois Agr. Exp. Sta., 1893 and 1894.

¹⁵ Hartley, C. P., "Progress in methods of producing higher yielding strains of corn," Yearbook, U. S. Dept. Agr., 1909, pp. 309-320, 4 pls.

¹⁶ *Op. cit.*

also reported on sixteen hybrid combinations all but two of which gave increased yields in the F_1 . From the work of all these men, especially from my own comparisons between F_1 and F_2 hybrids, it has become obvious that the secret of the highest success in corn breeding from an economic point of view lies in finding those strains which will produce the largest yield and then utilizing the first-generation hybrids each year.

The point which most interests us on the present occasion is not, however, the economic importance of using first generation crosses, but the evidence which appears to me clearly indicate that a normally cross-bred plant like Indian corn harmonizes in its fundamental nature with such normally self-fertilized material as beans, wheat and other grains, and such clonal varieties as potatoes, paramecium, etc., that the egg-cells and sperm-cells of even the most complex hybrids present a limited number of different types which can be assorted into homozygous combinations, and that, therefore, the progressive change resulting from continued selection may be simply explained as the gradual segregation of homozygous types or of the most efficient heterozygous combinations.

The fact that yield and perhaps many other qualities attain their highest development in the case of complex hybrids naturally leads to the unconscious selection of heterozygous plants for the next year's cultures, and the continual breaking up of these complex hybrids in subsequent generations gives a result which closely resembles fluctuating variation, but which is fundamentally different from it. The genuineness of the gains made by selection in corn might naturally lead to the conclusion that fluctuations are inherited were it not for the abundant evidence now available showing that a considerable portion of the variation presented is not fluctuational, but is due to the presence of a mixture of different types which any selection partially segregates.

NOTES AND LITERATURE

IS THE FEMALE FROG HETEROZYGOUS IN REGARD TO SEX-DETERMINATION?

THE evidence that sex is determined by an internal mechanism in unisexual animals has accumulated rapidly in the last few years. The one outstanding case is that of the frog. That extreme variations in the sex ratio occur in this amphibian has been evident from the early experiments of Born 1881, Pflüger 1882, and Yung 1883-85. The effects were generally ascribed by the earlier workers to differences in the food of the tadpole. Most recent and more carefully controlled experiments, notably those of Cuénot and of King, have shown beyond doubt that food is not a factor that determines the sex of the tadpole. On the other hand, Richard Hertwig has effected astonishing changes in the sex ratio of the frog by delaying fertilization of the eggs. Over-ripe eggs produce a high percentage of males. This conclusion has been recently confirmed and extended by a student of Hertwig's, Sergius Kuschakewitsch.¹ By delaying fertilization of the eggs for 89 hours after the first eggs had been laid (which gave 53 per cent. of males) there was produced 100 per cent. of males. The death rate of the larvæ was so low (from 4 to 6 per cent.) that it could not have seriously affected the results. The following table gives the outcome of Hertwig's observations and those of Kuschakewitsch.

Author	Hours 0	Hours 6	Hours 18	Hours 24	Hours 36	Hours 42	Hours 54	Hours 64	Hours 89
R. Hertwig, 1907	58%	54%	—	55%	—	—	87%	—	—
"	49	—	—	—	58%	—	59	—	—
"	48.5	—	37%	—	—	50%	—	88%	—
Kuschakewitsch	53	—	—	—	—	—	—	—	100%

This evidence shows beyond question that the *sex ratio* is affected by delay in fertilization, and may seem to show even that *sex itself* is determined by this factor. The evidence will, however, bear closer scrutiny. The frogs, *Rana esculenta*, were captured while pairing, and were allowed to lay a few eggs in

¹ Hertwig's Festschrift, 1910.

confinement, when they were separated. After 89 hours the female was killed, the remainder of her eggs placed on glass slides, and fertilized with a decoction of the testes of other (one or more?) males. If many of the eggs soon rotated within their membranes this was taken as a sign of successful fertilization. It will be noted that a different male from that employed for the normal fertilization was necessarily employed, because the original male had presumably lost his power to further fertilize. The employment of different males introduces a possible error into the results, for, if the male is heterozygous for sex determination, it is conceivable, as I have previously pointed out in reviewing Hertwig's results, that in different individuals the sperm may be differently affected in regard to its fertilization power. At present we have no evidence to show that in male frogs such differences exist, and it seems unlikely that such consistent results as these of Hertwig and of Kuschakewitsch can be explained in this way. An alternative view is, however, possible. If the female is heterozygous for sex production, and in consequence two kinds of eggs are produced, it may be that the female determining eggs are more injured by delay than are those of the other class, the male-determining eggs. It becomes, therefore, imperative to know what proportion of eggs were fertilized in these experiments. Unfortunately this critical evidence is omitted from Kuschakewitsch's paper. He states that the death rate of the tadpoles that emerge is low, but one looks in vain for information relating to the number of eggs that were fertilized. Therefore until this datum is forthcoming it is not possible to draw any certain conclusions in regard to sex determination from the evidence published by the author.

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THE MUTATION THEORY

The publication of the first volume of DeVries's "*Mutations-theorie*" in 1901, together with the rediscovery of Mendel's principles, served to bring about a period of unprecedented activity in the study of the problems connected with variation, heredity and evolution. While the results of this decade of work have probably raised as many questions as they have answered, yet the period has undoubtedly been marked by advances of the first importance, both in methods of investigation and in

results and the point of view achieved. This stimulus we owe in no small measure to the author of "Die Mutationstheorie." During this period DeVries himself has continued his activities uninterrupted except by his two visits to America, in which he did much through his letters and the publication of his "Species and Varieties" and "Plant Breeding," to familiarize his views to American biologists. However, the actual detailed data upon which his theory was based, remained largely a sealed book except to readers of German. Even those engaged in active work on these subjects frequently failed to acquaint themselves sufficiently with "Die Mutationstheorie" before breaking into the field of controversy. Particularly is this true of the second volume, the contents of which have been in large part neglected.

Professor Farmer and Mr. Darbishire have therefore performed an important service in translating this work into English. The first volume of their translation¹ is the subject of this review. The second volume is promised for April. The work will undoubtedly receive a wide reading by English-speaking biologists, and by others as well. The translation is an excellent one, faithful to the German meaning but rendered into idiomatic English. Whatever the degree of one's familiarity with the German edition, a perusal of the work in English will be found profitable and stimulating.

A few remarks regarding the contents of the book itself may not be out of place. In a re-perusal of the work, one is struck with the optimism of its author and with the brilliancy and breadth of his exposition of the views set forth. It is not necessary to agree with these views in their entirety in order to appreciate these qualities of the book. The analysis of the data amassed by Darwin, in which it is shown that Darwin's *single variations* are the same as De Vries's mutations, seems to the reviewer particularly effective. The conception of elementary species seems also one which will be of lasting value, having already shed a flood of light on many problems.

Probably the time will soon come when nearly all biologists will be ready to admit that mutation, or the sudden appearance of new forms, has been an important factor at least, in species formation in plants and animals. Admitting this, it remains to be discovered what relation these sudden appearances bear to the gen-

¹ DeVries, Hugo, 1909, "The Mutation Theory." Translated by Professor J. B. Farmer and A. D. Darbishire. Volume I. Six colored plates, figs. 119, pp. 582. Chicago, The Open Court Publishing Co.

eral trends of evolution, which are apparent in so many phylogenies. This larger problem, which may not be amenable to direct experimental attack, will probably occupy evolutionists for many years to come. For, granting the facts of mutation, we have only accounted for a micro-evolution, and it has still to be shown that the larger tendencies can be sufficiently accounted for by the same means, without the intervention of other factors.

While the supreme importance of DeVries's investigations on mutation in *Oenothera* is fully recognized, his premutation theory has always seemed to the reviewer unsatisfactory as a hypothesis to explain the material basis of these phenomena. The cytological investigations of myself and others on these forms have determined the events of germ cell formation, some of which provide a possible basis for the sudden appearance of new types. They have, moreover, shown that different cytological processes are involved in the origin of different mutants, and in this way have thrown much light on the relationships of some of the mutants to their parent form. It is probable that the whole question of the relation of the mutants to their parent will be found to be much more complex than at present supposed.

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